

Claims

1. A method for the amplification of genomic DNA whereby
5 the cytosine methylation pattern of the genomic DNA
is retained in the amplificate sequence(s), said
method comprising the following steps:
(a) heating the genomic DNA to a temperature opera-
tive to cause denaturation
10 (b) cooling the denatured DNA in the presence of sin-
gle stranded oligonucleotide primers such that the
primers anneal to the DNA
(c) heating the mixture in the presence of a poly-
merase and nucleotides to a temperature such that the
15 primers are extended
(d) contacting the double stranded nucleic acid with
a methyltransferase and a methyl donor molecule under
conditions conducive to the methylation of the syn-
thesised strand such that the CpG dinucleotides
20 within the synthesised strand are methylated accord-
ing to the methylation status of the corresponding
CpG dinucleotide on the template strand thereby pre-
serving the genomic methylation pattern
(e) repeating steps A-D a desired number of times to
25 reach a desired number of nucleic acids.
2. A method according to Claim 1 wherein the methyl-
transferase is a maintenance methyltransferase.
- 30 3. A method according to Claim 1 wherein
the methyltransferase is DNA (cytosine-5) Methyl-
transferase (DNMT 1).
4. A method according to Claims 1 to 3 wherein the
35 methyl donor molecule is S-adenosylmethionine.

5. A method according to Claims 1 to 4 wherein the methyl group carries a detectable label which is incorporated into the synthesised nucleic acid strand.
- 5 6. A method according to Claims 1 to 5 wherein a plurality of primer oligonucleotides are immobilised on a solid surface.
- 10 7. A method according to Claims 1 to 5 wherein the methyltransferase is immobilised on a solid surface.
8. A method according to Claims 1 to 5 wherein the polymerase is immobilised on a solid surface.
- 15 9. A method according to Claim 1 further comprising Step (f) a treatment with an agent capable of distinguishing between methylated and unmethylated cytosine bases.
- 20 10. A method according to Claim 9 wherein the agent is a methylation sensitive restriction enzyme.
11. A method according to Claim 9 wherein the agent is a bisulphite solution.
- 25 12. A device for the methylation pattern retaining amplification of nucleic acids according to Claim 1 said device comprising two or more reaction chambers, channel means providing fluid connections between adjacent chambers and the first and last reaction chambers, temperature regulating means for controlling the temperature of each reaction chamber.
- 30 13. A device for the methylation pattern retaining amplification of nucleic acids according to Claim 1 to 6 comprising
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two vessels,
a reaction chamber,
temperature regulating means for controlling the tem-
perature of the reaction chamber,
5 means for transferring liquid reagent from the first
and second vessels to the reaction chamber,
channel means providing fluid connections between ad-
jacent chambers and the first and last reaction cham-
bers
10 means for draining liquid reagents from the reaction
chamber.

14. A nucleic acid obtainable by a method according to
one of the claims 1 to 11.

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15. A method of manufacturing a methylated nucleic acid
using a method according to one of the claims 1 to
11.